8 ~~27.~~ (Amended) A protein according to claim 6, wherein said blocking group [has a molecular mass of approximately 43 atomic mass units] is an acetyl group.

Please add the following claim:

9 ~~28.~~ A protein according to claim 2, possessing a specific urate oxidase activity of about 30 U/mg.

REMARKS

Claims 1-7 and 27 are pending in the application. Claims 1-5 stand rejected under the first paragraph of 35 USC §112. The same claims are rejected under 35 USC §103 over Laboureur *et al.* ("Laboureur") in view of Reedy *et al.* ("Reedy") and Riggs or Neilsen *et al.* ("Neilsen"), in further view of Janson, Mannson-Rahemtulla *et al.* ("Mannson-Rahemtulla"), Nakagawa *et al.* ("Nakagawa") or Berton *et al.* ("Berton").

The claims have been amended to point out the invention more clearly and to claim it distinctly. For example, claim 1 has been revised to remove the recitation of homologues of the claimed protein. The recitation of specific activity of "at least 30 U/mg" has been removed from claim 2 and replaced with the recitation "wherein said protein is produced by recombinant methods." This amendment is supported generally in the specification at page 7, line 22 to page 10, line 31 and, in particular, at page 10, line 27. New claim 28, containing the specific activity recitation of former claim 2, has been added. Support for this claim derives from former claim 2 and page 4, lines 24-25, of the specification. Claim 3 has been amended to eliminate the multiple dependency and the recitation of "an isoelectric point around 8," since claim 5 includes a similar recitation. The multiple dependencies of claims 6 and 7 have

been deleted. The recitation of "a molecular mass of approximately 43 atomic mass units" has been deleted from claim 27 in favor of the more specific recitation of "an acetyl group." Support for this amendment is derived from page 4, lines 33-37.

37 CFR §1.75(c)

The examiner objects to claims 6, 7 and 27 as these are multiply dependent on other multiply dependent claims. Applicants have removed the multiple dependencies from claims 3, 6 and 7, thereby obviating the rejection.

35 USC §112, first paragraph

The examiner has rejected claims 1-5 on the grounds that the disclosure does not enable the range of all possible enzymes of "substantial homology" to the enumerated urate oxidase sequence of claim 1. While applicants traverse this rejection, claim 1 has been amended to remove the offending recitation, thereby advancing prosecution.

35 USC §103

The examiner has rejected claims 1-5 as obvious over Laboureur in view of Reedy and Riggs or Neilsen, in further view of Janson, Mannson-Rahemtulla, Nakagawa or Berton. Applicants have carefully reviewed the examiner's detailed comments and believe that the examiner has (i) failed to appreciate the relationship of the pseudo-affinity chromatography method to the cloning and expression of a recombinant protein and (ii) failed to attach the appropriate significance to the Larbre Declaration, describing the implementation of pseudo-affinity chromatography. Applicants provide the following comments in this regard.

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First, applicants wish to emphasize that three distinct urate oxidase (UO) preparations are under discussion here. The first preparation is that of Laboureur *et al.*, which exhibits a purity characterized by a specific activity of about 8 U/mg. The second preparation of UO is derived from natural sources purified by pseudo-affinity chromatography according to the present invention. See Example 4, specification at page 15. This form of UO exhibits a specific activity of at least about 16 U/mg. Finally, recombinant UO has any even higher purity, evidenced by a specific activity as high as about 30 U/mg. A proper appreciation of the difficulties associated with the production of the second and third preparations precludes any suggestion that these forms of UO are obvious over the Laboureur preparation in view of the supporting references.

In seeking a higher purity UO preparation, applicants found it necessary to conduct extensive experiments with both conventional and non-conventional purification methods. The declaration of Elisabeth Larbre provides an account of these experiments. After numerous failures, only pseudo-affinity chromatography procedures ***specifically modified for urate oxidase purification*** led to a UO preparation of a significantly greater purity, reflected in a specific activity of about 16 U/mg.

While the UO preparation described above is patentable over the Laboureur preparation (8 U/mg) in its own right (discussed below), it also served as a "stepping stone" in the acquisition of an even higher purity form of UO. Because the Laboureur preparation is of an insufficient purity to permit degradative sequencing, it would not be employed in the generation of nucleic acid probes based on partial amino acid sequences, for use in isolating the UO gene of *A. flavus*. Pseudo-affinity purified UO, however, is of sequenceable

quality. Following perfection of pseudo-affinity protocols, therefore, the protein sequence of *A. flavus* UO for the first time came within the reach of investigators.

Subsequently, probes were designed based on the inventors' sequence of pseudo-affinity purified UO and the *A. flavus* UO gene was cloned. Expression of the cloned UO gene according to the present invention results in a UO preparation with a specific activity as high as about 30 U/mg, greater than that of either the Laboureur preparation and natural, pseudo-affinity purified UO. Therefore, this preparation is patentably distinct over both the 8 U/mg and 16 U/mg preparations.

Turning to the examiner's commentary, applicants submit that the record contradicts each and every statement the examiner makes in his justification of the instant rejection. For example, the examiner states that "[i]t would have been obvious . . . to make *A. flavus* urate oxidase . . . by expressing the *A. flavus* urate oxidase gene as taught by Neilsen *et al.* or Riggs that had been isolated as taught by Reedy *et al.* . . . ." Thus, the examiner seems to believe that standard cloning approaches could have been applied to *A. flavus* UO. This view stands in stark contrast to (i) the examiner's admission that a high purity form of UO is of great interest to those of skill in the art and (ii) the failure of anyone to purify UO beyond the 8 U/mg reported by Laboureur in the seventeen years from the issuance of the Laboureur patent and the filing of the present application.

Moreover, it would have been impossible to isolate *A. flavus* UO using the methods of Reedy. In particular, the RNA extraction techniques used by Reedy, while suitably applied to rat liver cells, will not work with mycelium recovered from culture of *A. flavus*. Thus, neither the Reedy methodology nor

any modification of it which is suggested by the prior art can be considered as providing a reasonable expectation of success in the cloning of *A. flavus* UO. (Although they do not deem it necessary, applicants could oblige if the examiner were to conclude that a declaration elaborating on this point is critical.)

Also, the examiner states that "it would have been further obvious to purify the enzyme by means such as conventional, immunoaffinity or affinity chromatography." According to the examiner, while the Larbre Declaration "states that affinity chromatography was attempted...and did not result in the instantly claimed activities," the skilled artisan "would have known to vary...[the] experimental design to attempt to purify the enzyme." Applicants deem these statements to be in error for at least three reasons.

First, it appears the examiner simply doubts that the course of experimentation described in the Larbre Declaration was unpredictable when undertaken. If this is the examiner's position, it should be supported by more than the conclusory statement quoted above. In essence, the examiner must point to teachings in the prior art suggesting that the methods employed by applicants and some manner of modifications thereto (i) were within the purview of the skilled artisan at the time of the present invention and (ii) provided that artisan with a reasonable expectation of success in the endeavor of cloning *A. flavus* UO. Applicants submit, however, that the record is devoid of anything but the examiner's hindsight opinion in this regard. The examiner has never even mentioned pseudo-affinity chromatography, much less showed a reference that discloses anything like the methods described in Example 4. For this reason alone, the rejection must fall.

Second, the examiner states that it would have been obvious to "attempt to purify" UO according to the present invention. Applicants submit that this is an improper standard for judging the obviousness of the present invention. "Obvious to try" is not the standard for patentability in the U.S. Patent and Trademark Office. Again, this deficiency renders the entire rejection suspect.

Third, the examiner's comments reflect a crucial misunderstanding of the present invention. The examiner appears to believe that pseudo-affinity chromatography is applied to the recombinant product, thereby producing the claimed invention. In actuality, pseudo-affinity chromatography indirectly permits cloning and recombinant expression of UO. The use of pseudo-affinity chromatography further to purify recombinant UO is unnecessary because of the high quality of the starting material.

To summarize, applicants respectfully submit that it was not simply through modifying standard purification techniques that applicants arrived at their particular pseudo-affinity chromatography protocol. It was only after failure of standard methods and the implementation and modification of a new and untested approach, pseudo-affinity chromatography, that the 16 U/mg preparation was achieved. In addition, applicants stress that it was impossible to achieve the cloning of UO and, therefore, the high activity recombinant preparation, using standard cloning approaches like Riggs. Thus, only by using the pseudo-affinity purification regimen to achieve sequenceable grade UO was cloning of the UO gene made possible. Recognition of these facts makes withdrawal of the instant rejection an absolute necessity.

CONCLUSION

In light of the foregoing amendments and remarks, applicants submit that claims 1-7 and 27 are in condition for allowance and solicit an early indication to that effect. Should Examiner Schmickel believe that further discussion will advance the instant prosecution, he is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

May 10, 1993
Date

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